The thalassemias and related disorders

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The thalassemias, sickle cell disease, and other hemoglobinopathies represent a major group of inherited disorders of hemoglobin synthesis. The abnormal hemoglobins were reviewed in the July 2006 issue of *Baylor University Medical Center Proceedings*. Because of immigration patterns and population flow, these disorders are becoming increasingly more prevalent in the USA. In this article, the clinical aspects of the more common thalassemia syndromes are reviewed. For most symptomatic patients with thalassemia, there is no definite cure; only supportive management of the anemia is possible. A very limited number of patients with thalassemia may be cured by bone marrow transplantation from HLA-identical donors. Other tentative approaches to management include stimulation of fetal hemoglobin synthesis and attempts at somatic cell gene therapy. Prevention of disease transmission by carrier screening programs along with prenatal diagnosis remain of paramount importance in the reduction of these diseases worldwide.

The thalassemias are a group of anemias that result from inherited defects in the production of hemoglobin. The thalassemias are among the most common genetic disorders worldwide, occurring more frequently in the Mediterranean region (1), the Indian subcontinent, Southeast Asia, and West Africa (2). Ineffective bone marrow erythropoiesis and excessive red blood cell hemolysis together account for the anemia.

Since reticulocytes manufacture equimolecular quantities of alpha and beta chains, mature erythrocytes contain essentially equimolecular amounts of each chain (3). Patients with thalassemia do not produce enough hemoglobin (Hb) A ($\alpha_2\beta_2$) because their cells cannot manufacture either the alpha or beta polypeptide chain of human hemoglobin. Alpha-thalassemia depresses only the production of the alpha chains, and beta-thalassemia depresses only the production of the beta chains. Clinically, both alpha- and beta-thalassemia may occur in the major (homozygous), intermediate, and minor (heterozygous) genetic forms and also can interact with the presence of abnormal hemoglobins in the same individual (4–6).

To explain the nature of the thalassemia syndromes, it is necessary to outline the interplay of the various polypeptide chains of hemoglobin during normal human development. In the first trimester of intrauterine life, zeta, epsilon, alpha, and gamma chains attain significant levels and in various combinations form Hb Gower I ($\zeta_2\varepsilon_2$), Hb Gower II ($\alpha_2\varepsilon_2$), Hb Portland ($\zeta_2\gamma_2$), and fetal hemoglobin (HbF) ($\alpha_2\gamma_2$ 136-G and $\alpha_2\gamma_2$ 136-A) (7).

Whereas Hb Gower and Hb Portland soon disappear, HbF persists and forms the predominant respiratory pigment during intrauterine life. Before birth, gamma-chain production begins to wane so that after the age of 6 months postpartum, only small amounts of HbF (<2%) can be detected in the blood (8). In early intrauterine life, beta-chain synthesis is maintained at a low level but gradually increases to significant concentrations by the end of the third trimester and continues into neonatal and adult life. The synthesis of delta chains remains at a low level throughout adult life (<3%). Hence during normal development, the synthesis of the embryonic hemoglobins Gower and Portland is succeeded by the synthesis of HbF, which in turn is replaced by the adult hemoglobins, HbA and HbA_2 *(Figure)*.

Clinically the thalassemia syndromes are heterogeneous due to the many possible mutations affecting the human globin chain loci. These mutations include those of gene deletions, as well as globin chain initiation, translation, and termination (10, 11).

BRIEF HISTORICAL REVIEW

By the beginning of the 20th century, European clinicians had become aware of an anemia syndrome in infancy associated with enlargement of the spleen (12). In the American literature the first clinical description of thalassemia is attributed to the Detroit pediatricians Thomas B. Cooley and Pearl Lee (13). The actual term *thalassemia* was coined by George Whipple (14, 15). How this term arose remains obscure, although it is reported that early patients were mostly of Mediterranean origin.

During the 1960s a genetic basis of the thalassemia diseases was proposed, linking them to unbalanced globin chain synthesis (16, 17).

The stage was set for further progress. Simpler methodology was developed that made it possible for routine laboratories

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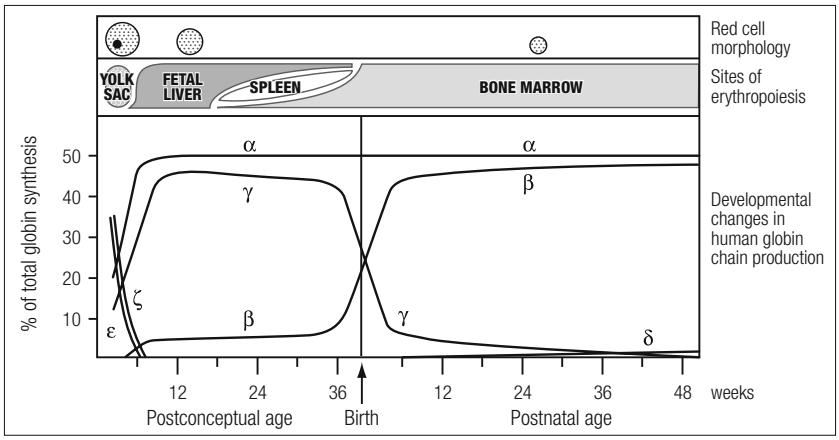

Figure. Developmental changes in human globin chain production, sites of erythropoiesis, and red cell morphology. Reprinted with permission from Wood WG, 1993 (9).

to analyze levels of hemoglobin A_2 and confirm the diagnosis of thalassemia (18). Other observations on the alterations of hemoglobin patterns in patients with thalassemia led to the discovery of HbH (β_4) (19) and Hb Barts (γ_4) (20), which later became established markers of alpha-thalassemia.

At Johns Hopkins University, David Weatherall and associates labeled reticulocytes of thalassemic patients with radioactive amino acids in vitro and were able to demonstrate that in patients with alpha- and beta-thalassemia, alpha- or beta-chain production was defective because of unbalanced globin chain synthesis (21).

At that point, it became necessary to determine whether protein synthesis was abnormal at the level of the structural gene or of globin chain synthesis. A series of experiments revealed a quantitative or qualitative deficiency of specific messenger RNA in many thalassemia syndromes as well as defects in the translation of the messenger RNA to protein (22). This latter stage requires ribosomal units that can initiate (promote or enhance), elongate, and terminate the globin chain (23).

Hence a clearer picture of the genetic control of human hemoglobins had emerged. It became clear that several structural loci, i.e., alpha, beta, gamma, and delta, were responsible for the production of their respective globin chains.

ALPHA-THALASSEMIA

Each human diploid cell contains four copies of the alpha-globin gene, located on chromosome 16. Whereas alpha-thalassemia is usually caused by one or more deletions of the alpha-globin chain loci, not all alpha-thalassemias are due to gene deletions (24). Clinically there are four alpha-thalassemia syndromes: silent carrier, alpha-thalassemia trait, HbH disease, and hydrops fetalis syndrome. These occur because of inheritance of molecular mutations affecting the output of one, two, three, or four of the alpha-globin genes *(Table).*

According to written convention, the alpha-thalassemia syndromes can be expressed as α^0 and α^+. In the α^0, no alpha chains are produced. In the α^+, the output of one of the linked pair of alpha-globin genes is defective, and only some alpha chains are produced. Within these general categories of the alpha-thalassemia syndromes, there is considerable genetic and clinical heterogeneity due to the interaction of the many possible mutations directing globin chain synthesis.

Since alpha chains are present in both fetal and adult hemoglobins, a deficiency of alpha-chain synthesis affects hemoglobin production in fetal as well as in adult life. A reduced rate of alpha-chain synthesis in fetal life results in the formation of gamma-chain tetramers (Hb Barts). In adult life, a deficiency of alpha chains results in the formation of beta-chain tetramers (HbH) as well as a deficiency in the formation of HbA_2 ($\alpha_2\delta_2$).

The anemia that ensues is also due to shortened red cell survival: beta-chain tetramers (HbH) can precipitate and form inclusion bodies that damage the red cell membrane. Since Hb Barts (γ_4) is more stable than HbH (β_4), it does not readily form inclusion bodies. Nevertheless, both Hb Barts and HbH show no heme-heme interaction and have high oxygen affinities. Consequently, they are extremely poor oxygen carriers (25).

In response to the oxygen deprivation caused by the anemia, the dyserythropoietic marrow expands, leading to extramedullary erythropoiesis in the bone, liver, and spleen. This erythropoiesis gives rise to skeletal deformities and bony fractures, megaloblastic anemia due to folate deficiency, and hyperuricemia with gout.

GLOBIN CHAIN TERMINATOR CODON MUTATIONS

The term *codon* represents the sequence of three bases that determines the specificity of an amino acid to be included in a polypeptide chain. Several mutations that give rise to elongated chains have been described in the terminator codon of both the alpha- and beta-chain genes. The first elongated globin was discovered in a Chinese family in Jamaica and was called Hb Constant Spring (Hb CS). The alpha chain in Hb CS has 172

Table. Alpha-thalassemia syndromes

Syndrome	Number of alpha-globin genes affected	Clinical features	Hemoglobin pattern
Silent carrier (α^+)	1	No or minimal anemia	1%–2% Hb Barts (γ_4)
Thalassemia trait (α^+)	2	Mild anemia Hypochromic microcytic	5%–10% Hb Barts (γ_4)
HbH disease ($\alpha^0 + \alpha^+$)	3	Moderate anemia Hypochromic microcytic RBC inclusion bodies	10%–30% HbH (β_4)
Hydrops fetalis (α^0)	4	Death in utero or at birth Severe anemia	97% Hb Barts (γ_4) 3% HbH (β_4)

RBC indicates red blood cells.

instead of 141 amino acids (26). Also, there is a marked reduction of alpha-globin RNA in the red cells of homozygous Hb CS individuals (27). Similar mutations that give rise to elongated beta-chain globin have been described. The Hb TAK beta chain consists of 157 amino acids instead of 146 (28).

BETA-THALASSEMIA

The beta-gene cluster region resides on chromosome 11. The beta-thalassemias can be divided into several varieties. In β^0 thalassemia, there is a total absence of beta-chain production. In β^+ thalassemia, there is a partial deficiency of beta-chain production. Hypochromia and microcytosis characterize all forms of beta-thalassemia.

Because the synthesis of beta chains is almost completely inhibited in thalassemia major, a severe anemia begins at about 3 to 6 months of age, the time when gamma-chain synthesis normally decreases. The anemia produces a stress situation in the bone marrow. This leads to the continuation of HbF synthesis but at a rate far below what is necessary for adequate compensation of the anemia. The HbF produced is unevenly distributed in the red cells and accounts for the anisochromasia. Large numbers of imperfect red cells are destroyed in the bone marrow, giving rise to ineffective erythropoiesis, which is such a prominent feature of the disease. Accelerated apoptosis, the major cause of ineffective erythropoiesis, is caused by excess alpha chain deposited in the erythroid precursors (29).

The hemoglobin pattern in patients with homozygous thalassemia (beta-thalassemia major) consists of a variable increase in HbF, which then accounts for 8% to 90% of the total hemoglobin concentration. The terms *beta-thalassemia intermedia, beta-thalassemia minor,* and *beta-thalassemia trait or carrier* are used to reflect the decreasing clinical severity of the anemia.

DELTA-BETA-THALASSEMIA

In $\delta\beta^+$ thalassemia, an abnormal hemoglobin, Hb Lepore, is produced (30). Hb Lepore has a normal alpha chain combined with a nonalpha chain that consists of the N-terminal residue of the delta chain fused with the C-terminal residue of the beta chain. Many different varieties of Hb Lepore have been described in which the transition from delta to beta amino acid sequences occurs at different points (25). Essentially the Lepore nonalpha chain is a delta-beta fusion chain.

This hemoglobin has little clinical importance yet is of great genetic interest. The occurrence of Hb Lepore serves to establish the concept of neighboring delta- and beta-chain loci on the same gene, with the delta locus leading the beta locus (31). Individuals who carry such genes have up to 25% Hb Lepore in their circulation as well as increased levels of HbF (5%–70%). The Lepore hemoglobins have been found sporadically in most racial groups and rarely give rise to significant anemia.

HEMOGLOBIN E BETA-THALASSEMIA

Compound heterozygotes for HbE and beta-thalassemia are extremely common in Thailand and Southeast Asia. Patients with these disorders can suffer from a moderate to severe anemia and require regular transfusion (2). Today HbE thalassemia is considered one of the most common and most important hemoglobinopathies in the world. This condition is becoming more prevalent in the USA as a result of Asian immigration.

HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN

Hereditary persistence of fetal hemoglobin (HPFH) is a state in which the pattern of hemoglobin production of the unborn child continues into adult life. The switching on of the beta- and delta-chain loci does not take place, and the adult continues producing gamma chains. No deleterious effects are apparent, even when 100% of the hemoglobin synthesized is HbF ($\alpha_2\gamma_2$). More than 40 examples of the heterozygote state of HPFH have been described to date. In these individuals, about 15% to 30% of the hemoglobin is HbF. HPFH becomes clinically important when it is inherited together with beta-thalassemia or the sickle-cell gene. In such cases, HPFN's increased output of HbF ameliorates the degree of the anemia and acts as a protective agent against sickling.

Increased levels of HbF may also occur in various disorders such as juvenile chronic myeloid leukemia, Blackfan-Diamond anemia, Fanconi anemia, and paroxysmal nocturnal hemoglobinuria. Many of the conditions resulting in increased HbF in adult life appear to involve an increased erythropoietic drive, which results in a higher proportion of erythroid progenitor cells activating their inherent ability to synthesize some amounts of HbF. Treatment of sickle cell anemia with hydroxyurea may increase fetal hemoglobin over 20%, ameliorating the anemia with improvement in the clinical manifestations of the disease. An excellent review of HbF increases in adult life has been published (9).

CLINICAL FEATURES OF SEVERE THALASSEMIC SYNDROMES

Infants and children affected with thalassemia have pallor, poor development, and abdominal enlargement. Hemoglobin electrophoretic patterns show a variable quantity of HbA_2 (0%–6%) depending on the genotype of the patient. The anemia is due to a combination of ineffective erythropoiesis, excessive peripheral red blood cell hemolysis, and progressive splenomegaly (25). The latter causes an increase in plasma volume and a decrease in total red cell mass. The reticulocyte count is usually <1%. The red cells are microcytic (mean corpuscular volume <70 fL) with marked anisochromasia. The bone marrow shows marked erythroid hyperplasia, and the serum ferritin level is elevated. For diagnostic purposes the parents' hematologic status should be evaluated.

In children and young adults, radiologic abnormalities include thinning of the long bones with sun-ray appearance and dilation of the marrow cavities. The skull has a "hair-on-end" appearance because of widening in the diploic space. Patients with thalassemia have enlarged maxillary sinuses and tend to have a maxillary overbite. The face gradually assumes a "mongoloid" appearance. Such changes promote infections in the ears, nose, and throat. Because of chronic anemia and iron overload, endocrinopathies such as hypopituitarism, hypothyroidism, hypoparathyroidism, diabetes mellitus, cardiomyopathy, and

testicular or ovarian failure become common as the child with thalassemia grows older (32, 33).

Thalassemia can be regarded as a chronic hypercoagulable state (34). Venous and arterial thromboembolic phenomena tend to occur more frequently in thalassemic patients who have undergone splenectomy. Furthermore, such patients may develop progressive pulmonary arterial disease due to platelet thrombi in the pulmonary circulation. The reasons for the procoagulant effect in thalassemia remain obscure, although it has been proposed that erythrocyte membrane abnormalities such as phosphatidylserine formation on the surface of thalassemic red cells activate the coagulation system.

THERAPEUTIC MEASURES

Chronic anemia in thalassemic patients is managed with blood transfusion, iron chelation, and splenectomy in cases of hypersplenism. In recent years several different transfusion regimens have been proposed to promote normal growth, decrease cardiac load, and lessen iron deposition in tissues. More recently, clinical experience suggests that such criteria may be achieved by maintaining a hemoglobin level of 9 to 10 g/dL throughout life (35). The transfusions are given once a month using washed, filtered, or frozen red cells in order to reduce noxious reactions to foreign cells and plasma proteins. A few medical centers specializing in the treatment of thalassemic patients use younger red cell populations, "neocytes," for transfusion (36) with the aim of reducing transfusion frequency.

Prevention of hemosiderosis

Hemosiderosis in thalassemia is secondary to frequent transfusions and increased iron absorption associated with ineffective erythropoiesis. One unit of packed red cells contains about 250 mg of iron, and some patients receive >100 units of packed red blood cells. Because no mechanisms exist for increasing iron excretion (except for phlebotomy), thalassemic patients are set up for developing hemosiderosis. Iron deposition is ubiquitous and occurs in the skin, endocrine organs, liver, spleen, and heart. Cardiac toxicity and pump failure is a frequent cause of death. Hence, iron overload causes most of the mortality and morbidity associated with thalassemia (37).

Nontransferrin-bound iron (low-molecular-weight iron) is a highly toxic state of iron formed when the iron-binding capacity of transferrin has been exceeded (38). Hence, long-term transfusion programs should be accompanied by therapy with iron-chelating agents within the first 3 years of life (39). At present deferoxamine is widely used for iron chelation. However, because it is poorly absorbed from the gastrointestinal tract, it must be administered intravenously or subcutaneously via a metering pump. Deferoxamine gives rise to neurosensory toxicity in about 20% of cases, causing high-frequency hearing loss. Color and night blindness have also been reported. Physicians prescribing deferoxamine should perform auditory and ophthalmic examinations biannually.

Ferritin levels below 2500 mg/mL are associated with improved survival (40). However, ferritin levels tend to be unreliable in the presence of liver disease, and then liver biopsy may be indicated. It is important to try to maintain serum ferritin levels below 1500 mg/mL.

Ascorbic acid supplementation in small doses (≤100 mg/day) will increase iron chelation with deferoxamine. However, it can generate free radicals with resultant cardiac toxicity. Finally, the oral iron chelator deferasirox (Exjade) is now widely used and well tolerated except for mild gastrointestinal toxicity. This drug is well absorbed with hepatobiliary excretion of chelated iron into the gut rather than the urinary excretion noted with deferoxamine.

Stimulating HbF production

Individuals with HPFH demonstrate that preventing or reversing the switch from fetal to adult hemoglobin would provide efficacious therapy for thalassemia and various other hemoglobinopathies. It has been observed that some patients recovering from cytotoxic therapy have reactivated HbF synthesis. Several therapeutic agents such as erythropoietin, hydroxyurea, cytarabine, and butyrate analogs have produced an increase in HbF synthesis in the thalassemic patients by stimulating the HbF-producing progenitor cell population (41, 42).

Bone marrow transplantation

To date, over 1000 bone marrow transplants have been performed in thalassemic patients at medical centers of excellence (43). Disease-free survival of 80% to 90% was achieved in patients who had adequate iron chelation and did not suffer from liver failure or hepatomegaly. In patients without these good prognostic features, disease-free survival rates dropped to 50%.

Somatic gene therapy

Human globin genes have been transferred into mouse cells (44). Successful application of gene transfer for the treatment of thalassemia is experimental and will require that the newly introduced genes do not alter the growth properties of the bone marrow cells by the recombinant retroviral genome (45). Design of vectors that ensure adequate production of globin messenger RNA to correct the deficiency in thalassemic red cells requires more experimentation.

Prevention

In countries with a high incidence of thalassemia, it is vitally important to offer prospective genetic counseling and to warn carriers about the risks of intramarriage. To date, attempts at this approach have been relatively unsuccessful. Hence, considerable efforts have been directed towards prenatal diagnosis programs. As carrier states of the thalassemias are readily identifiable, affected fetuses can be diagnosed. Recent efforts have been directed to early diagnosis by fetal DNA analysis (46) carried out on amniotic fluid cells or by chorionic villus sampling. Also, the development of oligonucleotide probes to detect individual mutations has markedly increased the accuracy rate of prenatal diagnosis. The harvesting of fetal cells from the maternal circulation is being explored for this purpose (47).

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